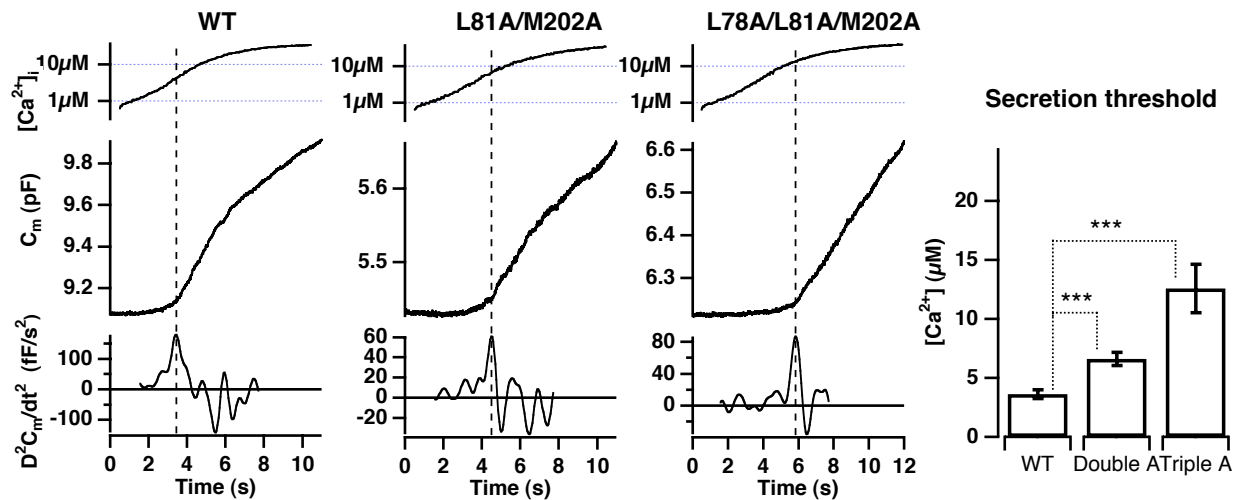


## SUPPLEMENTARY FIGURE 2



Suppl Fig. 2. Calcium ramp stimulation of double and triple mutations in layers 7 and 8.

The double (L81A/M202A) and triple (L78A/L81A/M202A) mutations were studied alongside with WT rescued cells by stimulation with  $Ca^{2+}$ -ramps (top panels). The capacitance increase (middle panels) was measured during slowly increasing calcium concentrations, and after appropriate smoothing the second derivative of the capacitance trace was used to find the point of maximal capacitance acceleration (bottom panel). This value (termed secretion threshold), was increased in the double mutation, and even more so in the triple mutation (right panel). This experiment indicates a graded effect of accumulating mutations in the C-terminal end of the SNARE complex. N=13 (WT), N=12 (L81A/M202A) and N=8 (L78A/L81A/M202A) cells.